

GHESQUIERE et al
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26. (Amended) A fragment of less than about 5cM of chromosome 4 and a polymorphous AFLP band according to claim 15 which includes the RYMV resistance locus.

REMARKS

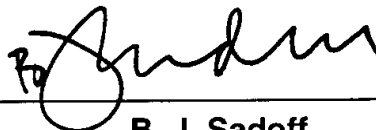
The above amendments are made to place the claims in a more traditional format.

Submitted herewith is the executed Declaration and the filing fee.

Respectfully submitted,

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MARKED UP CLAIMS

1. (Amended) Method for identifying markers of the locus of a major resistance gene to RYMV, comprising:

[- selective amplification of] selectively amplifying rice DNA fragments firstly from resistant individuals, and secondly from sensitive individuals, descending from parental varieties, these fragments being previously subjected to a digestion step, then a ligation step to fix complementary primer adapters having at their end one or more specific nucleotides, one the primers of the pair being labelled for development purposes, to produce amplification products,

[- separation of] separating the amplification products, by gel electrophoresis under denaturing conditions, to produce electrophoresis profiles and

[- comparison of] comparing the electrophoresis profiles obtained with mixtures of fragments derived from resistant descendants and mixtures derived from sensitive descendants, with fragments derived from parental varieties, for the purpose of identifying bands whose polymorphism is genetically linked to the resistance locus, this identification optionally being followed, for validation purposes, by verification on each individual and calculation of the genetic recombination rate between the marker and the resistance locus.

2. (Amended) Method according to claim 1, [characterized in that] wherein the DNA fragments are obtained by digestion of the genomic DNA of resistant plants and of sensitive plants, and their parents, using restriction enzymes.

3. (Amended) Method according to claim 2, [characterized in that as] wherein said restriction enzymes are at least one of EcoRI and MseI [are used].

4. (Amended) Method according to claim 2[or 3], [characterized in that] wherein the restriction fragments are [subjected to ligation to fix] ligated to adapters.

5. (Amended) Method according to claim 4, [characterized in that] wherein the fragments obtained are amplified using primer pairs complementary to the adapters, whose sequences are respectively GAC TGC GTA CCA ATT C(SEQ ID N°1) and GAT GAG TCC TGA GTA A(SEQ ID N°2).

6. (Amended) Method according to claim 4[or 5], [characterized in that] wherein the fragments obtained are amplified using primer pairs having at their end the respective motifs AAC and CAG, ACC and CAG, and optionally also [or further] AGC and CAG.

7. (Amended) Method according to [any of claims 1 to 6, characterized by the identification of] claim 1, further comprising identifying resistance marker bands, M1 and M2, whose size is respectively 510 bp and 140 bp[, such as determined by gel electrophoresis under denaturing conditions].

8. (Amended) Method according to claim 7, [characterized in that] wherein said marker bands determine a segment of less than 10-15 cM carrying the resistance locus.

9. (Amended) Method according to claim 8, [characterized in that] wherein said marker bands are located either side of the locus at less than 5-10 cM.

10. (Amended) Method according to [any of claims 1 to 9, characterized in that it also comprises an isolation step to isolate the] claim 7, further comprising isolating said identified marker bands.

11. (Amended) Method according to claim 10, [characterized by purification of] further comprising purifying the isolated marker bands in order to obtain DNA fragments.

12. (Amended) Method according to claim 11, [characterized by] further comprising cloning [of] the marker bands into a vector and insertion of the vector in a host cell.

13. (Amended) Method according to [either of claims 11 or 12, characterized by the recovery] claim 11, further comprising recovering and sequencing [of] the purified, cloned DNA fragments.

14. (Amended) Method for obtaining markers having high specificity for the locus of a major RYMV resistance gene, comprising cloning and amplifying cloned fragments of said locus with [characterized in that] PCR primer pairs [are defined] which are complementary to the sequence of the cloned fragment, [specific amplification of this fragment is carried out using these primer pairs, then the amplification products are subjected] and subjecting amplification products to migration on an electrophoresis gel with or without previous digestion of said amplification products by a restriction enzyme, to identify a polymorphism.

15. (Amended) A Polymorphous AFLP band [bands such as] identified by the method according to [any of claims] claim 1 [to 14] using rice plant DNA.

16. (Amended) A AFLP [bands] band according to claim 15, [characterized in that they are specifically evidenced in] wherein said rice plant is a RYMV-sensitive variety, or [and in the fraction of] a plant which is the progeny of an RYMV-sensitive [plants derived from crossing of this variety with] plant and a resistant variety.

17. (Amended) A DNA [sequences] sequence corresponding to a polymorphous [bands] band according to claim 15 or 16, which can be used to define a segment of chromosome 4 of 10-15 cM carrying the RYMV resistance locus.

18. (Amended) A DNA [sequences] sequence according to claim 17, [characterized in that they correspond to] which is an EcoRI-MseI [fragments] fragment.

19. (Amended) A DNA [sequences] sequence according to claim 18, [characterized by a respective size of] which is between 510 bp and 140 bp, determined by gel electrophoresis under denaturing conditions.

20. (Amended) A DNA [sequences] sequence according to [any of claims 17 to 19, characterized in that they correspond to sequences] claim 17 which is a sequence flanking the resistance locus [and located either side of the latter at 5-10 cM or even at less than 5 cM] within a distance of 10 cM.

21. (Amended) A DNA sequence of, characterized in that it meets] SEQ ID N°3 or SEQ ID N°9.

23. (Amended) A cloning vector [Cloning vectors, characterized in that they contain] comprising at least one of sequence SEQ ID N°3 according to claim 21 [or sequence] and SEQ ID N°9 [according to claim 22].

24. (Amended) A host cell [Host cells, characterized in that they are] transformed by a vector [vectors] according to claim [22] 23.

25. (Amended) Use of polymorphous bands according to claim 15 [or 16 or of DNA sequences according to any of claims 17 to 22] for the identification of resistant phenotypes and transfer of the resistance gene.

26. (Amended) A fragment of less than about 5cM [Fragments of no more than 4-5cM] of chromosome 4 and a polymorphous AFLP [bands] band according to claim 15 [or 16 defining a segment of 4-5cM or less carrying] which includes the RYMV resistance locus.